

Dynamics of Liver Stiffness Values by means of Transient Elastography in Patients with HCV Liver Cirrhosis undergoing Interferon Free Treatment

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ABSTRACT

Background & Aims: Liver stiffness (LS) measurement by Transient Elastography (TE) has been widely accepted as a tool for fibrosis assessment. The aim of this study was to assess LS dynamics in a group of patients with HCV liver cirrhosis after interferon free treatment (IFT).

Methods: This two-center clinical trial included 225 patients with compensated HCV cirrhosis (all genotype 1b), who received IFT for 12 weeks. All patients were evaluated by means of TE at the beginning and at the end of treatment (EOT), and a subgroup (170 patients) also 12 weeks after EOT; all of them had sustained viral response (SVR). Reliable LS measurements (LSM) were defined as a median value of 10 valid LSM, with IQR <30% and SR ≥60%. Both M and XL probes were used. For diagnosing cirrhosis we used a cut-off value of 12kPa as proposed by the Tsochatzis meta-analysis. We considered a decrease or increase of more than 10% in LSM as being significant.

Results: Out of 225 subjects, reliable measurements were obtained in 93.7%, so that the final analysis included 211 patients. The mean LS values decreased significantly after IFT: 26.4±11.7 vs. 23.5±13.3 kPa (p=0.01). Most patients, 59.2% (125/211) presented more than 10% decrease in LS values, 24.1% (51/211) had stable LS values, while in 16.4% (35/211) cases, the LS values increased. In the subgroup of 170 patients with LSM also performed 12 weeks after EOT (SVR), the mean LS values were significantly lower as compared to baseline: 21.3±11 kPa vs. 27.4±11.9 kPa (p<0.0001) and also as compared to EOT: 21.3±11 kPa vs. 23.7±13.3 kPa (p<0.0001).

Conclusion: In our patients with HCV liver cirrhosis, the mean LS values evaluated by TE significantly decreased after antiviral treatment at EOT and also 12 weeks after EOT as compared to baseline. Overall, about 60% of patients had LS values at EOT lower than at baseline, while 12 weeks after EOT about 75% of patients had LS values lower than at baseline.

Key words: liver stiffness – liver cirrhosis – HCV – interferon free treatment – end of treatment.

Abbreviations: APRI: aspartate aminotransferase platelet ratio index; BMI: body mass index; DAA: direct acting agents; EOT: end of treatment; FIB-4: fibrosis 4; HBV: hepatitis B virus; HCV: hepatitis C virus; IFT: interferon free treatment; IQR: interquartile range; LS: liver stiffness; LSM: liver stiffness measurements; SR: success rate; SVR: sustained viral response; TE: Transient Elastography.

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INTRODUCTION

Hepatitis C virus (HCV) chronic infection is a worldwide problem, with more than 180 million subjects infected around the world [1]. Following an acute infection, more than 70% of the patients remain chronically infected and the disease, while in many cases silent, can progress to liver cirrhosis. Very often,

subjects infected with HCV 20 or 30 years before, are discovered with advanced cirrhosis or even with hepatocellular carcinoma (HCC).

For approximately 20 years, the only treatment of HCV chronic infection was interferon based (first with standard interferon and later with pegylated interferon) in combination with ribavirin. In the last few years, new treatments for HCV infection were developed, the so-called direct acting agents (DAA) or interferon free treatment (IFT). The advantages of such drugs are the high success rates (more than 90%), the short duration of the treatment (12 or 24 weeks) and the few side effects.

One of the most important questions that arise is what happens with liver fibrosis following a sustained virologic response (SVR). Many published studies have shown a partial reversibility of fibrosis during treatment or after SVR in HCV or HBV chronic infection [2-8]. Post treatment evaluation of liver fibrosis was performed only by liver biopsy for a long time. This is the reason why few longitudinal studies evaluated this aspect: only two or rarely three liver biopsies have been prelevated post treatment, because few patients accept serial liver biopsies, especially when the viral infection was healed.

Starting more than 10 years ago, the severity of liver fibrosis was assessed by ultrasound-based elastographic measurement of liver stiffness (LS). Transient elastography (TE) was the first ultrasound-based elastography technique used in clinical practice, and current EASL guidelines recommend this method for the evaluation of patients with chronic liver diseases [2, 10]. This noninvasive method measures LS and a result is available in less than 5 minutes. Several papers and meta-analyses demonstrated the value of TE in HCV chronic hepatitis and cirrhosis [11-14]. It is repetitive, painless and not very expensive. Considering the good performance of this method, the post-treatment follow-up of patients with HCV chronic hepatitis can be easily done.

However, when considering TE as a non-invasive tool for fibrosis assessment we must mention that not only fibrosis influences LS values. A previous study that assessed TE considering liver biopsy as a reference method, demonstrated that LS is a sum of fibrosis and inflammation, and that it also might be influenced by liver steatosis [15]. There are also several confounding factors that can lead to higher LS values which might overestimate fibrosis: sinusoidal pressure [16], inflammation, as suggested by high aminotransferase levels [17, 18], extrahepatic cholestasis [19], liver congestion due to heart failure [20]. The influence of inflammation can be supported by the observation that LS decreases in patients with alcoholic liver disease following alcohol withdrawal [21], and also in patients with chronic hepatitis C following HCV clearance [22-24]. A significant decrease of LS values was observed both in animal models and in human subjects after removing obstruction of the main bile duct in subjects with obstructive jaundice [19]. The diuretic treatment in patients with congestive heart failure leads to a decrease in LS values [20]. Many other factors may also increase LS such as hepatic infiltration with tumor cells, inflammatory cells, mastocytosis or amyloidosis [18].

Thus, the post treatment LS decrease can be the result of the attenuated inflammation, and/or of a decrease in fibrosis severity. The question arises whether LS should be evaluated very early, or later post treatment, and how much later. The aim of the present study was to assess the dynamics of LS by means of TE in patients with HCV liver cirrhosis, before and immediately after IFT.

MATERIAL AND METHODS

Patients

The investigation was performed in accordance with the Helsinki Declaration. Written informed consent was given by all the study participants. Individuals older than 18 years

of age, with HCV liver cirrhosis were enrolled. Pregnant women and patients with unreliable TE measurements were excluded.

This two-center clinical trial was performed in two University Departments of Gastroenterology and Hepatology and included 225 consecutive patients previously diagnosed with compensated HCV liver cirrhosis, based on clinical, biological, ultrasonographic, morphologic, laparoscopic or endoscopic (esophageal varices) criteria, who underwent IFT (Viekirax/Exviera + Ribavirin) over a period of 12 weeks, starting from December 2015 until September 2016. All cirrhotic patients were infected with HCV genotype 1b. In all subjects FibroTest was performed at baseline, for a double certification of cirrhosis and for necro-inflammation grading. Cirrhosis was defined as compensated, if Child-Pugh class A, no ascites on transabdominal ultrasound and a total bilirubin <2mg/dL. All patients were screened for HCC by means of performant liver ultrasound.

Transient elastography

Liver stiffness was measured by means of TE using FibroScan® device (EchoSens, Paris, France), which incorporates an ultrasound transducer probe mounted on the axis of a vibrator. All the patients were evaluated by means of TE at the beginning (baseline) and at the end of treatment (EOT), all of them being responders. A subgroup of our cohort was also evaluated by TE 12 weeks after EOT.

Measurements were made in the right liver lobe, according to the methodology described in current guidelines [10, 25-27]. Reliable TE measurements were defined as median values of 10 valid LS measurements, with interquartile range IQR <30% and success rate SR ≥60% [10, 25-27]. Both M and XL probes were used. For the diagnosis of liver cirrhosis we used a cut-off value of 12kPa, as suggested in the Tsochatzis meta-analysis [14]. We defined as being significant a decrease or an increase of more than 10% in LS values as compared to the beginning of treatment.

Clinical and biological assessment

Clinical parameters were collected the same day when TE measurements were performed. We recorded the following data: age, gender, body mass index (BMI), previous response to therapy. Biochemical parameters and blood count were collected at each visit (baseline, EOT, 12 and 24 weeks after EOT). The FibroTest was performed in all patients at baseline.

Statistical analysis

The Kolmogorov-Smirnov test was used for testing the distribution of numerical variables. Qualitative variables were presented as numbers and percentages. Parametric tests (*t*-test, ANOVA) were used for the assessment of differences between numerical variables with normal distribution; and nonparametric tests (Mann-Whitney or Kruskal-Wallis tests) for variables with non-normal distribution. Chi-square (χ^2) test (with Yates' correction for continuity) was used for comparing proportions expressed as percentages ("n" designates the total number of patients included in a particular subgroup). 95% confidence intervals were calculated for each predictive test and a *p*-value <0.05 was considered as significant for all

statistical tests. The statistical analysis was performed using SPSS software, Version 20.0 (IBM SPSS Statistics), Microsoft Office Excel 2007 and GraphPad Prism version 5.

RESULTS

Out of 225 subjects, reliable measurements by TE were obtained in 93.7%, so that the final analysis included 211 patients (116 women and 95 men), mean age 59.2±8.7 years, BMI 27.4±4.3kg/m². The characteristics of the patients are presented in Table I.

Table I. Characteristics of the 211 patients evaluated in the study

	n (%)
Age (years)*	59.2±8.7*
Gender	
- male	116 (54.9%)
- female	95 (45.1%)
Mean body mass index, BMI (kg/m ²)*	27.4±4.3
Previous exposure to therapy	
- Naïve	100 (47.3%)
- Non-responders to IFN based treatment	111 (52.7%)

*mean ± standard deviation, n=number of patients

At EOT, the mean LS values of the study group significantly decreased as compared to baseline: 23.5±13.3 kPa (95%CI: 21.2-23.9) vs. 26.4±11.7 kPa (95%CI: 24.9-27.5) (p=0.01) (Figs. 1, 2).

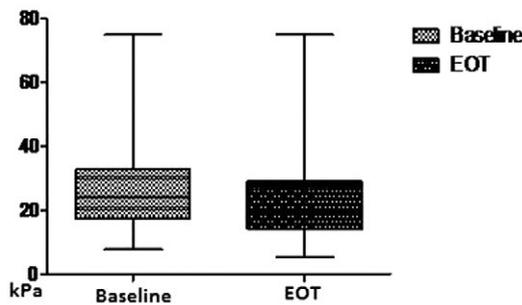


Fig. 1. Mean liver stiffness values by TE at baseline and at end-of treatment (EOT)

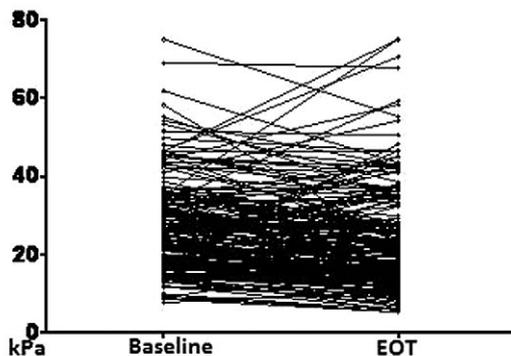


Fig. 2. Dynamics of liver stiffness values from baseline to the end-of treatment (EOT).

Almost 60% of patients (59.2% - 125/211) presented more than 10% decrease in LS values; in 24.1% (51/211) the LS values remained stable, while in 16.4% (35/211) of cases, the LS values increased. The absolute values of LS changes are shown in Fig. 3.

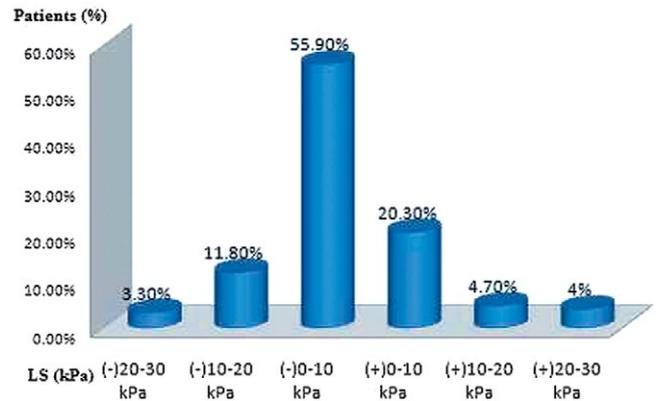


Fig. 3. Liver stiffness changes in the studied group

We compared the patients' characteristics in the three groups (decreased, stable and increased LS), and we found no significant differences regarding age, gender or BMI (Table II).

Table II. Characteristics of the patients according to liver stiffness (LS) values

Parameter	Decreased LS (n=125)	Stable LS (n=51)	Increased LS (n=35)	p value
Age (years)	58.9±8.2	59.6±9.3	59.61±8.4	0.71
Gender	F=69 (55.2%) M=56 (44.8%)	F=32 (62.7%) M=19 (37.3%)	F=19 (54.2%) M=16 (45.8%)	0.48 0.95
BMI (kg/m ²)	27.6±4.3	26.5±4.6	27.8±3.7	0.79

F=female, M=male

Regarding necro-inflammation, at baseline the activity score as assessed by FibroTest tended to be significantly higher in the group with decreased LS values at EOT as compared to the group with stable or increased LS values at EOT, 0.67±0.1 (95%CI: 0.51-0.75) vs 0.64±0.2 (95%CI: 0.50-0.69) (p=0.05).

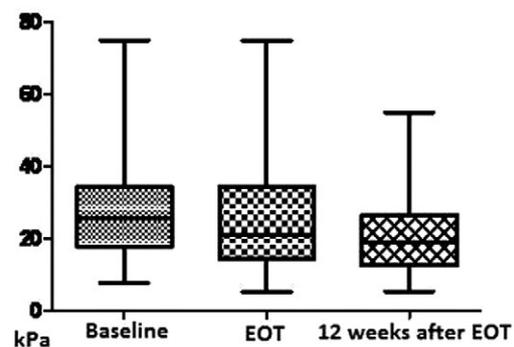


Fig. 4. Mean liver stiffness values by TE at baseline, end-of treatment (EOT) and 12 weeks after EOT

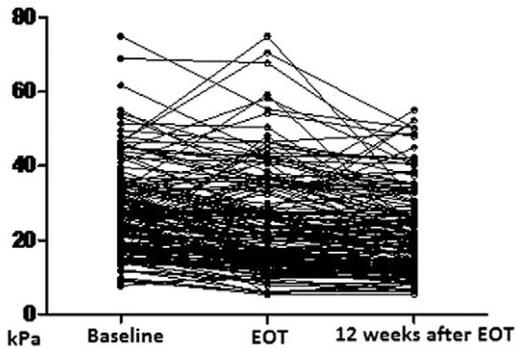


Fig. 5. Dynamics of liver stiffness values from baseline, to end-of-treatment (EOT) and 12 weeks after EOT.

Evaluating other factors that can also be associated with a decrease of LS values, both in univariate and multivariate analysis, alanine aminotransferase (ALT) was significantly correlated with the decrease of LS ($p=0.001$). Factors such as BMI, age, or aspartate aminotransferase (AST) level were not correlated with the decrease of LS ($p=0.6$, $p=0.76$, $p=0.53$, respectively).

In a subgroup of 170 patients (all with SVR) we also performed LS measurements at 12 weeks after EOT. The mean LS values were significantly lower after EOT as compared to baseline: 21.3 ± 11 kPa (95%CI: 20.1-24.9) vs. 27.4 ± 11.9 kPa (95%CI: 25.2-29.6) ($p<0.0001$) (Figs. 4, 5) and also as compared to EOT: 21.3 ± 11 kPa (95%CI: 20.1-24.9) vs 23.7 ± 13.3 kPa (95%CI: 23.5-24.2) ($p<0.0001$) (Fig. 6).

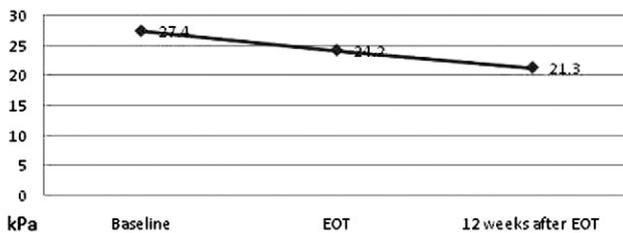


Fig. 6. Evolution of mean liver stiffness values in the studied group

Three quarters of the patients (75.2% - 128/170) presented more than 10% decrease in LS values 12 weeks after EOT as compared to baseline; in 13% (22/170) of cases the LS values remained stable, while in 11.8% (20/170) cases they increased.

DISCUSSION

Noninvasive techniques are very tempting for the dynamic evaluation of liver fibrosis over time, since they are repetitive, atraumatic, inexpensive and well accepted by the patients. But can we evaluate only fibrosis, or more changes, such as inflammation and maybe also steatosis by TE? To partially solve this question, we designed this study, in which after 12 weeks of treatment we evaluated LS in HCV patients with viral response. Since the follow-up period was short, only 12 weeks after treatment, most probably not fibrosis regression was responsible for LS decrease, but improvement of inflammation. Therefore, the elastographic follow-up of cirrhotic patients who achieved SVR should start from the values measured at EOT or

12 weeks after EOT (when SVR is assessed), so we could find out how much fibrosis has decreased in this advanced liver disease.

Several studies have been published regarding the LS values assessed by TE during and after treatment, both in HCV and HBV chronic infection. In a study that assessed LS values by TE before and after interferon based treatment in a cohort of 76 HCV patients, it was found that in the 55 patients with SVR, the LS values at EOT significantly decreased as compared to baseline (6.8 ± 4.9 kPa vs. 9.5 ± 6.9 kPa, $p=0.04$) [28]. The decreased LS values were maintained in the subgroup of patients who were followed-up 3 years later.

In another study [29], which evaluated LS values by TE in HCV patients treated by pegylated interferon and ribavirin, followed-up for at least 18 months, it was found that LS values decreased during time in patients with SVR, as compared with those without SVR. A multivariate logistic analysis of this cohort showed that high γ -GT values and histological steatosis were independently associated with a persistence of higher values of LS.

In a French study, in which LS values and liver biopsy were performed during the follow up of HCV infected patients with SVR, the LS values were lower than 12 kPa in 38% of the patients who had cirrhosis on liver biopsy. The authors concluded that the predictive power of the viremic cut-off of 12 kPa for LS is low in patients with SVR due to liver re-modelling and fibrosis resorption [30].

A limit in the follow-up of these patients by TE can be the low reproducibility of LSM in some cases [31].

Other researchers assessed the evolution of fibrosis in HCV patients after SVR using biological tests (FibroTest) [32]. A cohort of HCV patients was followed up for 10 years after SVR, a regression of fibrosis being defined as a minimum 0.20 decrease in the Fibrosis score on FibroTest. From the 415 patients with baseline advanced fibrosis, in 49% of the 108 patients with SVR a regression of fibrosis was found. In the cohort of cirrhotic patients, cirrhosis regressed in 24/43 patients.

A German study evaluated a cohort of 392 HCV patients treated with IFT [8]. Transient elastography values, APRI score and FIB-4 were recorded prior to IFT and at 18 months after therapy. A significant decrease of about 32% was observed of the median TE values, from 12.65 kPa to 8.55 kPa ($p<0.001$). Also median FIB-4 and APRI values significantly decreased. In conclusion the authors asked themselves if the rapid decrease of TE measurements, which was in concordance with the significant decrease in the biologic fibrosis scores was linked entirely to a decrease of fibrosis, or it was also due to the resolution of the inflammation following SVR [8].

A similar decrease of TE measurements, APRI score and FIB-4 was observed in an Egyptian study following a Sofosbuvir-based IFT. In univariate and multivariate analysis, failure to improve TE measurements following treatment was associated with relapse and with low baseline LS values [9].

The short duration of IFT in HCV compensated cirrhosis (12 weeks) makes the decrease of fibrosis improbable; most probably, the decrease of LS values as compared to baseline was the result of inflammation attenuation. We consider that EOT is a reliable moment to start fibrosis follow-up by TE, every 6 months. On the other hand, all the cirrhotic patients must be followed-up by ultrasound every 6 months, for HCC

screening, having in mind that this risk is still present, despite viral eradication.

A limit of this study could be the absence of liver biopsy as a reference method. But serial biopsies (before and after treatment) are quite rarely performed and usually not in large cohorts of patients. Serial LS measurements by TE during longer follow-up periods are missing, but this will be our next objective.

CONCLUSION

In our patients with SVR, the mean LS values evaluated by TE significantly decreased after antiviral IFT as compared to the baseline values, both at EOT and also 12 weeks after EOT. Overall, almost 60% of the patients had lower LS values at EOT, as compared to baseline values, while 12 weeks after EOT (SVR) almost 75% of patients had LS values lower than at baseline.

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Authors' contributions: I.S. wrote the manuscript; I.S., A.P., R.S. and L.G. designed and supervised the study; R.L., R.M. and S.I. performed the research; R.L. and R.S. analyzed the data. All authors prepared and revised the manuscript, and agreed to the final version of the manuscript.

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